

Supplemental Material

Extracellular vesicles mediate the horizontal transfer of an active LINE-1 retrotransposon

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Supplemental Figures

Figure S1. Representative photos of exosomes from HCT116, HCT116 L1-EGFP, MM231, and MM231 L1-EGFP cells taken by TEM.

Figure S2. L1 RNA abundance and RNP complex accumulation are critical and rate-limiting for L1 retrotransposition.

Figure S3. Expression of L1-encoded proteins in recipient cells.

Figure S4. Image of NHDF cells co-cultured with MM231 L1-EGFP cells using a 1.0 μ m transwell for 14 days.

Figure S5. APOBEC3 genes are upregulated in L1-EGFP cell lines.

Figure S6. Uncropped Western blot images for Fig. 3C.

Figure S7. Uncropped Western blot images for Fig. 5D and 5E.

Figure S8. Uncropped Western blot images for Fig. S3.

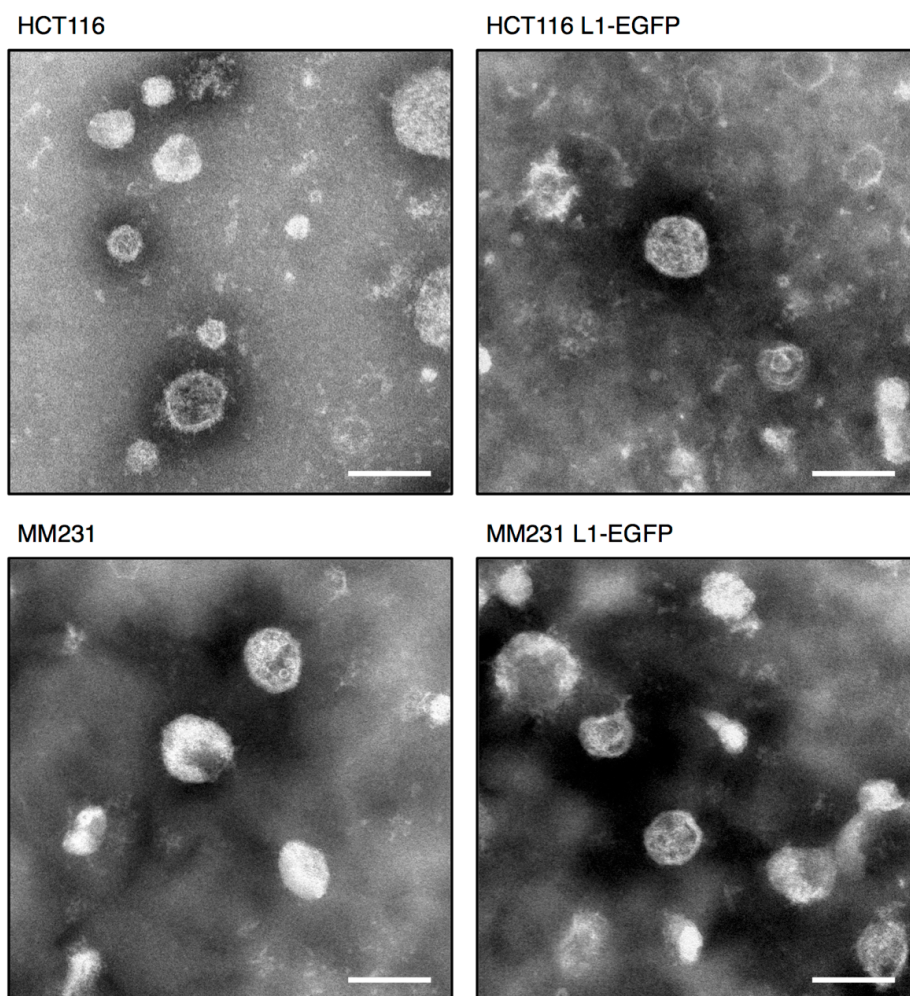


Figure S1. Representative photos of exosomes from HCT116, HCT116 L1-EGFP, MM231, and MM231 L1-EGFP cells taken by TEM. Exosomes were prepared from the culture supernatant of cells by filtration through a 0.22 μm filter followed by ultracentrifugation at $210,000 \times g$. Scale bars represent 100 nm.

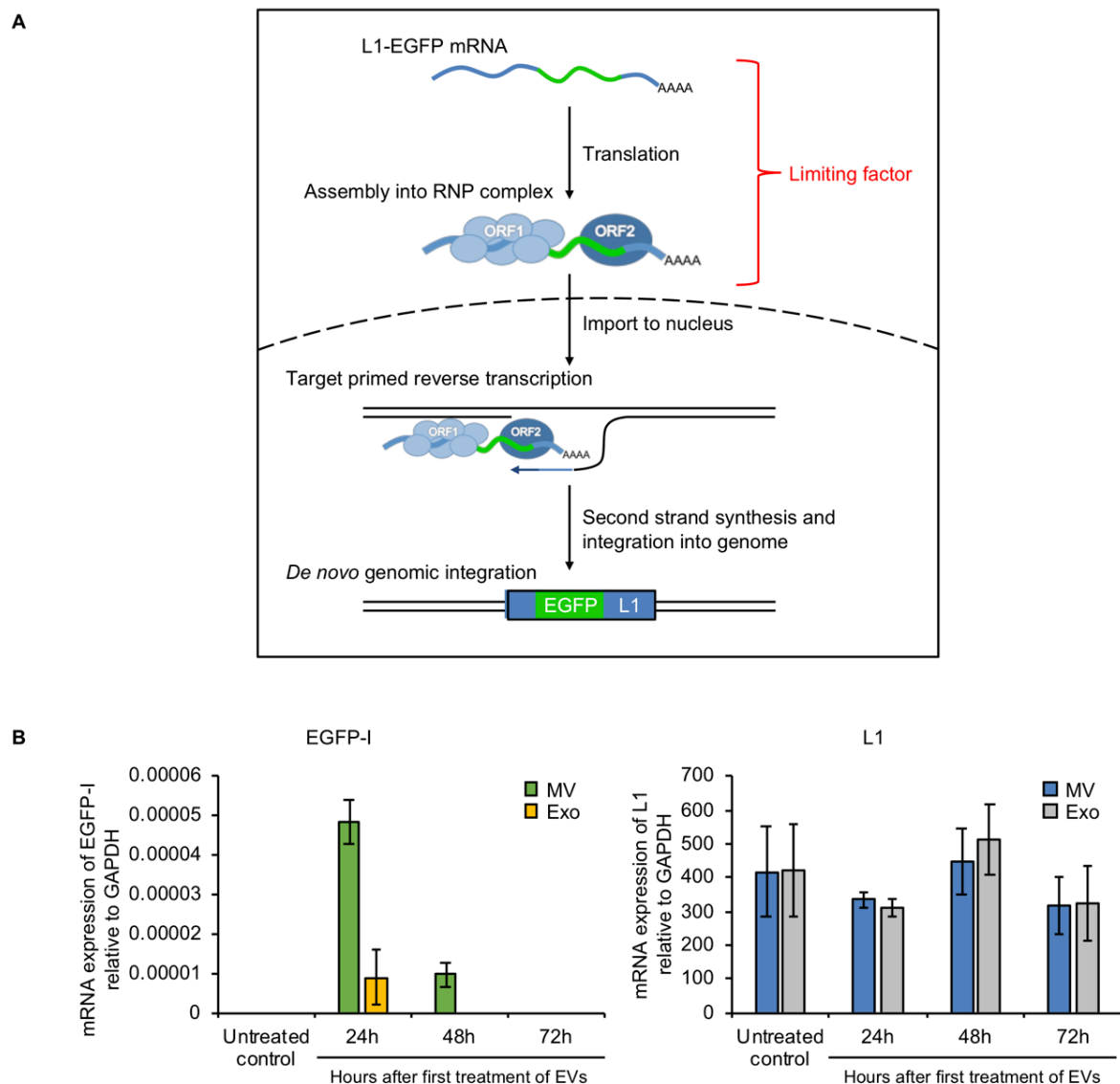


Figure S2. L1 RNA abundance and RNP complex accumulation are critical and rate-limiting for L1 retrotransposition. (A) Schematic illustration of L1-EGFP retrotransposition. ORF1 and ORF2 proteins are translated and bind to the L1 RNA to form the L1 ribonucleoprotein (RNP) complex. The RNP complex is imported to the nucleus where the RNA intermediate is reverse transcribed and integrated into the genomic target site. (B) mRNA expression of EGFP-I and L1 relative to GAPDH in untreated cells and cells treated with L1-EGFP MVs or exosomes. Total RNA was collected from cells after 24, 48, and 72 hours after the first treatment with L1-EGFP MVs or exosomes. $N=3$ wells in 6-well plate. Error bars represent SD.

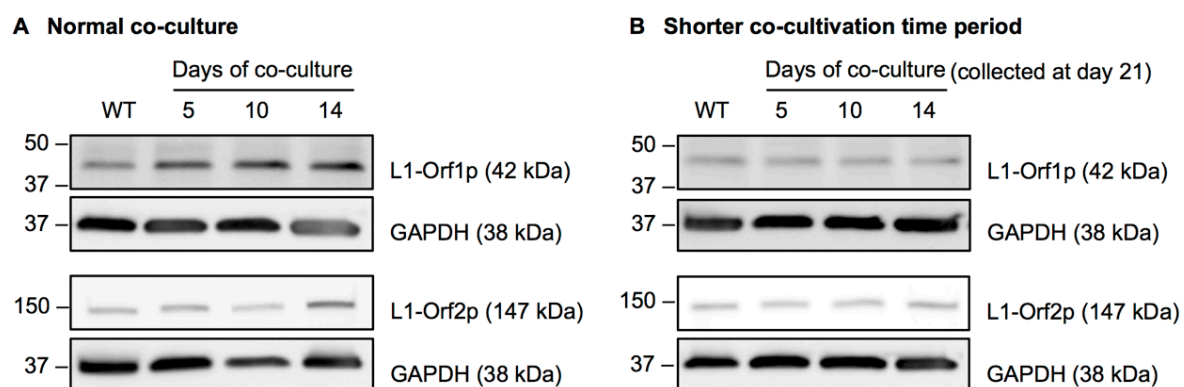


Figure S3. Expression of L1-encoded proteins in recipient cells. MM231 cells were co-cultured with MM231 L1-EGFP cells for 5, 10, 14, or 21 days prior to removal of the transwell insert containing MM231 L1-EGFP cells. After removal of MM231 L1-EGFP cells, recipient cells were cultivated until day 21. (A) Whole cell lysates of MM231 cells and MM231 cells continuously co-cultured with MM231 L1-EGFP cells for 5, 10, or 14 days were separated on SDS-PAGE gels, followed by Western blotting using antibodies against L1-Orf1p, L1-Orf2p and GAPDH. (B) Whole cell lysates of MM231 cells and MM231 cells co-cultured with MM231 L1-EGFP cells for 5, 10, or 14 days and cultivated until day 21 were separated on SDS-PAGE gels, followed by Western blotting using antibodies against L1-Orf1p, L1-Orf2p and GAPDH. Equivalent amount of protein (20 μ g) was loaded for each sample.

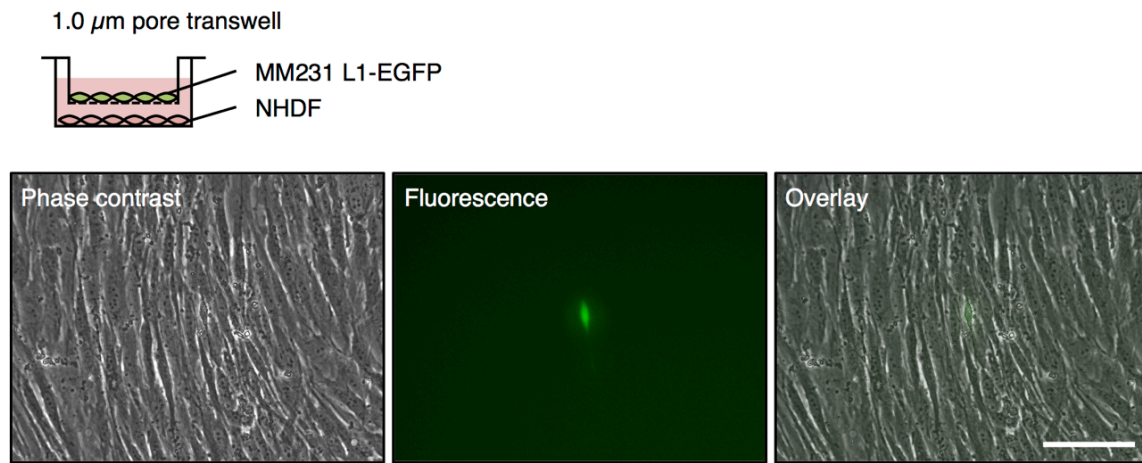


Figure S4. Image of NHDF cells co-cultured with MM231 L1-EGFP cells using a 1.0 μm transwell for 14 days. Scale bar represents 200 μm .

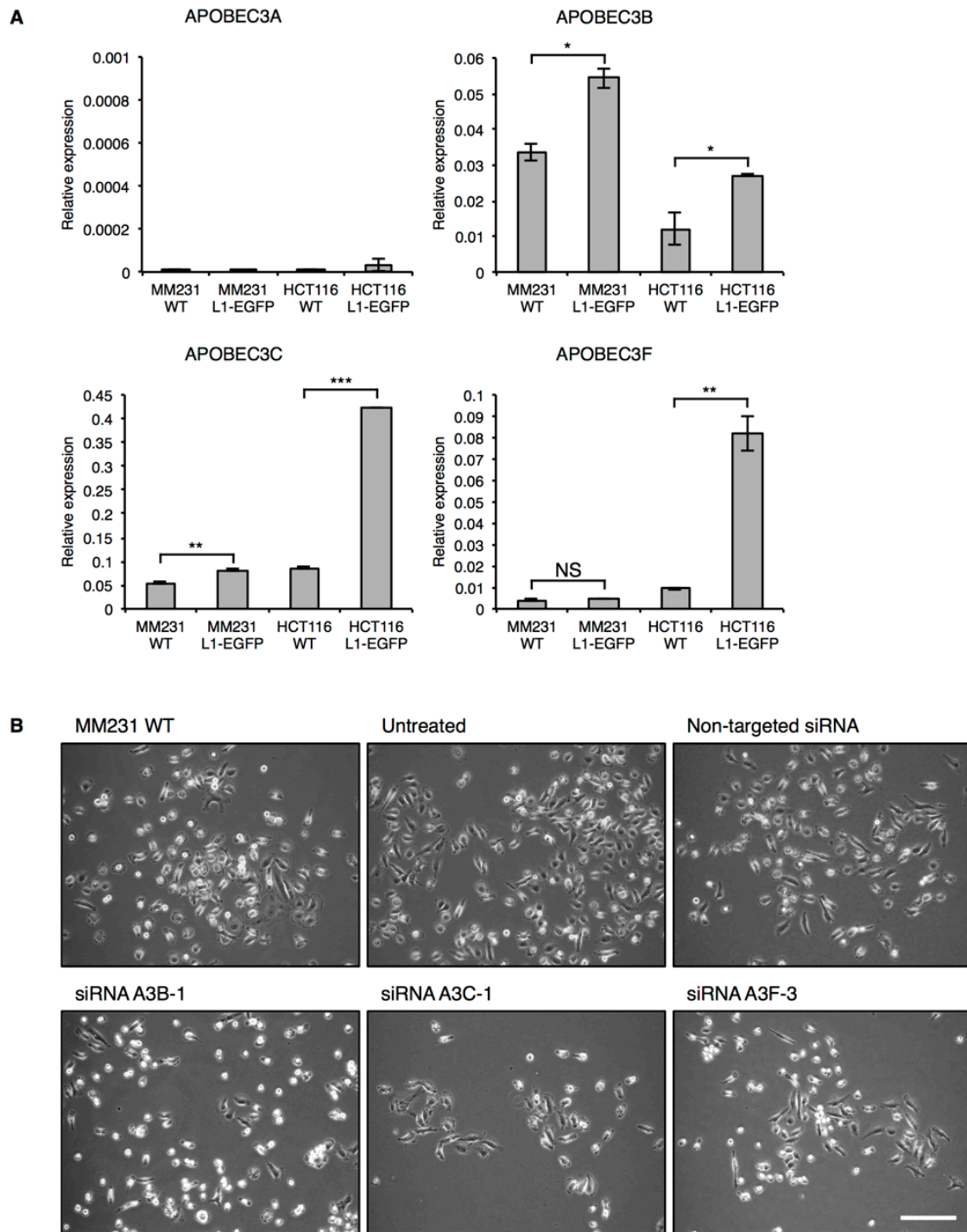


Figure S5. APOBEC3 genes are upregulated in L1-EGFP cell lines. (A) qRT-PCR for A3B, A3C, and A3F in cells transfected with the L1-EGFP construct. RNA levels were normalized to GAPDH. $N=3$ wells in 6-well plate. Error bars represent SD. *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$; t -test. (B) Images

of MM231 cells 3 days after transfection with non-targeted siRNA, A3B-1, A3C-1, and A3F-3 siRNA.

Scale bar represents 200 μm .

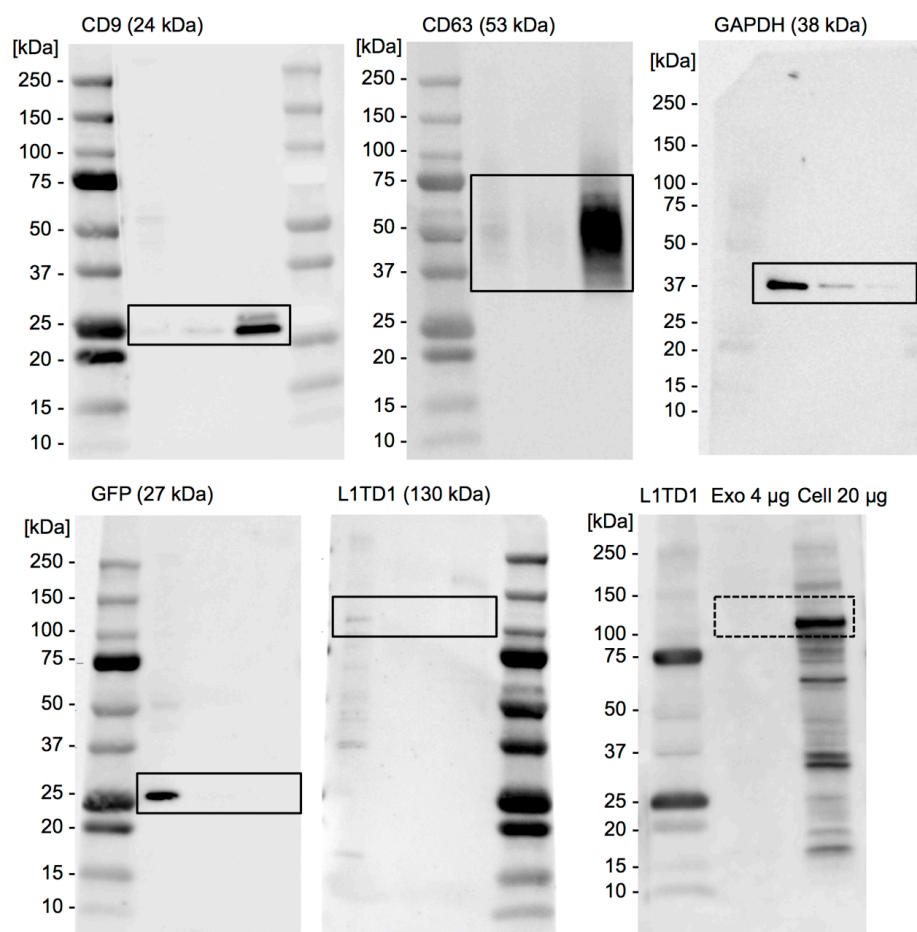


Figure S6. Uncropped Western blot images for Fig. 3C.

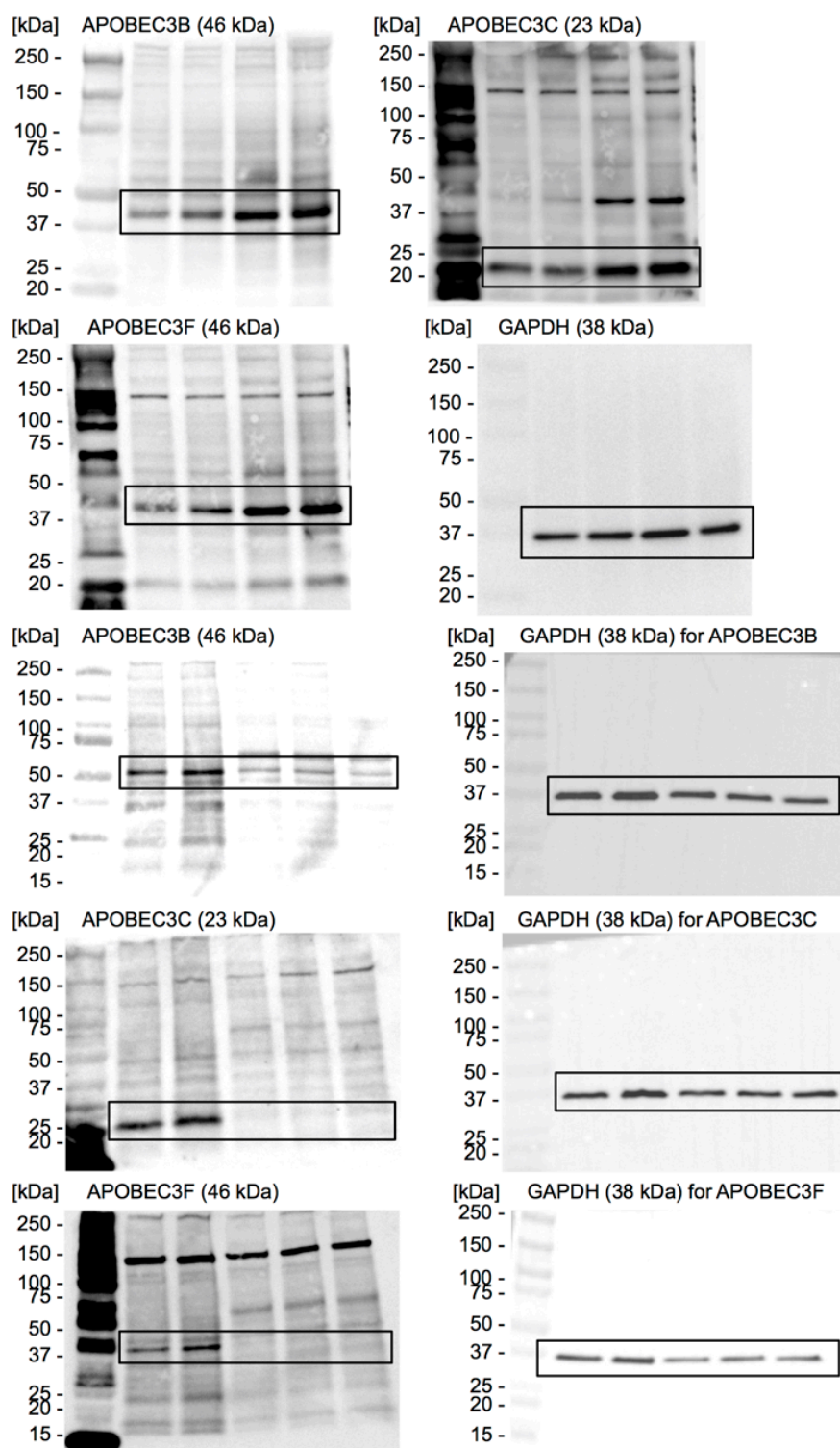


Figure S7. Uncropped Western blot images for Fig. 5D and 5E.

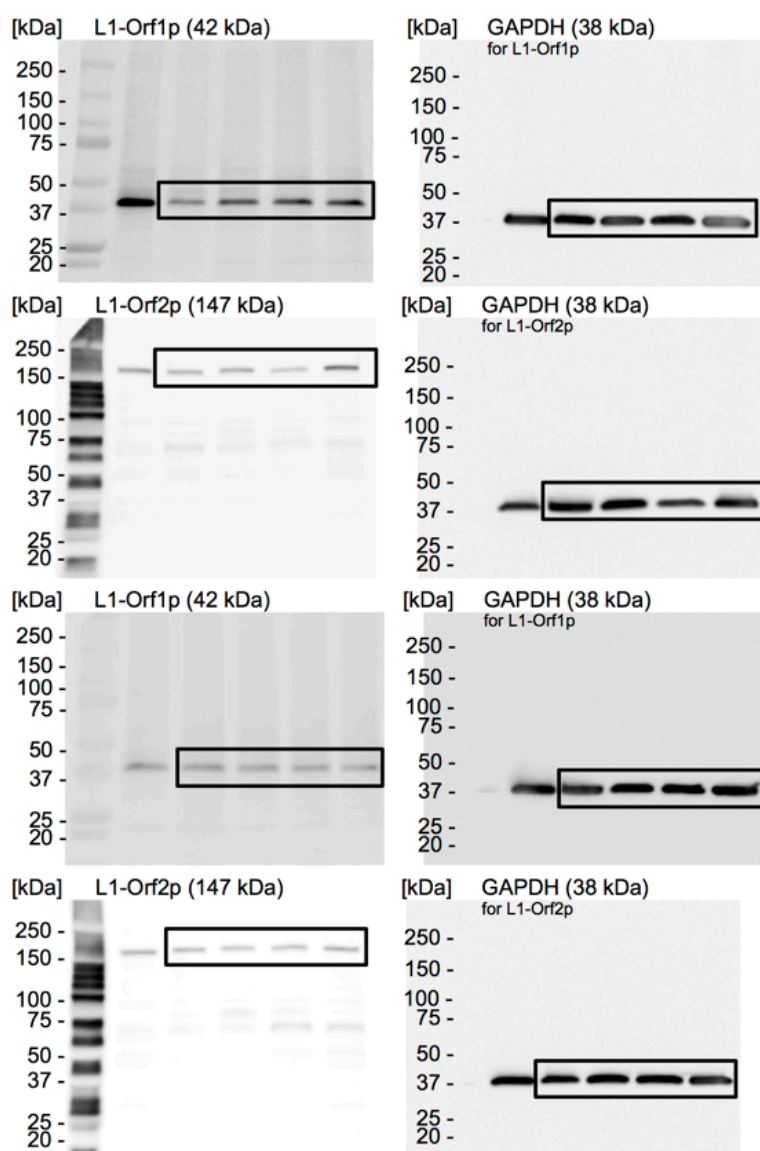


Figure S8. Uncropped Western blot images for Fig. S3.